NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF EMODIN

(CAS NO. 518-82-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

June 2001

NTP TR 493

NIH Publication No. 01-3952

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP reports printed since 1982 appears on the inside back cover.

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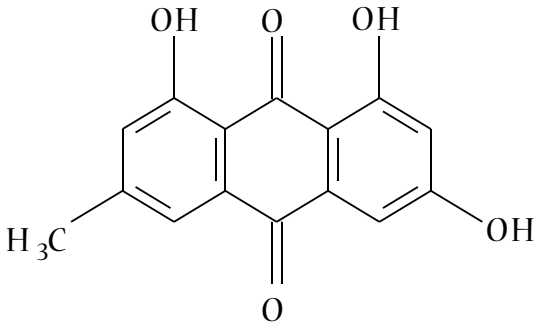
CONTENTS

ABSTRACT



EMODIN

CAS No. 518-82-1

Chemical Formula: $C_{15}H_{10}O_5$ Molecular Weight: 270.23

Synonyms: Archin; C.I. 75440; C.I. Natural Green 2; C.I. Natural Yellow 14; emodol; frangulic acid; frangula emodin; 6-methyl-1,3,8-trihydroxyanthraquinone; Persian Berry Lake; rheum emodin; schuttgelb; 1,3,8-trihydroxy-6-methyl-9,10-anthracenedione; 1,3,8-trihydroxy-6-methylanthraquinone; 4,5,7-trihydroxy-2-methylanthraquinone

Emodin is a naturally occurring anthraquinone present in the roots and bark of numerous plants of the genus *Rhamnus*. Extracts from the roots, bark, and/or dried leaves of buckthorn, senna, cascara, aloe, frangula, and rhubarb have been used as laxatives since ancient times and currently are widely used in the preparation of herbal laxative preparations. Anthraquinone glycosides are poorly absorbed from the gastrointestinal tract but are cleaved by gut bacteria to produce aglycones (such as emodin) that are more readily absorbed and are responsible for the purgative properties of these preparations. There is extensive exposure to emodin and other anthraquinones resulting from the use of herb-based stimulant laxatives. Reports that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumors in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin is a hydroxyanthraquinone structurally similar to 1,8-dihydroxyanthraquinone, is present in herbal laxatives, and was reported to be mutagenic in bacteria, it was considered a potential carcinogen and was selected for in-depth evaluation. Male and female F344/N rats and B6C3F$_1$ mice were exposed to emodin (at least 94% pure) in feed for 16 days, 14 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, rat and mouse bone marrow cells, and mouse peripheral blood erythrocytes.

16-DAY STUDY IN RATS

Groups of five male and five female rats were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 50, 170, 480, 1,400, or 3,700 mg emodin/kg body weight to males and 50, 160, 460, 1,250, or 2,000 mg/kg to females) for 15 (males) or 16 (females) days. Three female rats died before the end of the study. Mean body weights of males and females exposed to 5,500 ppm or greater were significantly less than those of the controls. Feed

consumption by males and females receiving 17,000 or 50,000 ppm was decreased throughout the study. Macroscopic lesions were present in the kidney of rats exposed to 17,000 or 50,000 ppm.

16-DAY STUDY IN MICE

Groups of five male and five female mice were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 120, 400, 1,200, or 3,800 mg/kg to males and 140, 530, 1,600, or 5,000 mg/kg to females; 50,000 ppm equivalents were not calculated due to high mortality) for 15 (males) or 16 (females) days. All mice exposed to 50,000 ppm died before the end of the study. Mice in the 17,000 ppm groups lost weight during the study. Feed consumption by 5,500 ppm females was greater than that by the controls throughout the study. Macroscopic lesions were present in the gallbladder and kidney of mice exposed to 17,000 ppm.

14-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 20, 40, 80, 170, or 300 mg/kg to males and females) for 14 weeks. Mean body weights of males exposed to 2,500 ppm or greater and females exposed to 1,250 ppm or greater were significantly less than those of the controls. During the first week of the study, feed consumption by males exposed to 2,500 or 5,000 ppm and females exposed to 5,000 ppm was less than that by the controls. Feed consumption by these groups was similar to that by the controls for the remainder of the study. In rats exposed to 2,500 or 5,000 ppm, there were increases in platelet counts in males and females and segmented neutrophil counts in females. Total serum protein and albumin concentrations were decreased in females exposed to 2,500 or 5,000 ppm. Relative kidney weights of rats exposed to 1,250 ppm or greater and relative lung weights of rats exposed to 625 ppm or greater were significantly increased compared to the control groups. Relative liver weights were increased in females exposed to 625 ppm or greater. The estrous cycle length was significantly increased in females exposed to 1,250 or 5,000 ppm.

All male rats exposed to 1,250 ppm or greater and all exposed female rats had pigment in the renal tubules; and the severity of pigmentation generally increased with increasing exposure concentration. The incidences of hyaline droplets in the cortical epithelial cytoplasm were increased in all groups of exposed males and in females exposed to 312.5, 625, or 1,250 ppm.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 50, 100, 190, 400, or 800 mg/kg to males and 60, 130, 240, 500, or 1,100 mg/kg to females) for 14 weeks. All mice survived to the end of the study. Mean body weights of males exposed to 2,500 or 5,000 ppm were significantly less than those of the controls. Feed consumption by exposed groups was generally similar to that by the controls. Relative kidney weights of male mice exposed to 1,250 ppm or greater, relative lung weights of males exposed to 625 ppm or greater, and relative liver weights of female mice exposed to 625 ppm or greater were increased.

The incidences and severities of nephropathy were increased in males and females exposed to 1,250 ppm or greater. The incidences of renal tubule pigmentation were significantly increased in males exposed to 625 ppm or greater and in females exposed to 1,250 ppm or greater.

2-YEAR STUDY IN RATS

Groups of 65 male and 65 female rats were fed diets containing 0, 280, 830, or 2,500 ppm emodin (equivalent to average daily doses of approximately 110, 320, or 1,000 mg/kg to males and 120, 370, or 1,100 mg/kg to females) for 105 weeks. Ten male and ten female rats from each group were necropsied at 6 months. Blood samples from five male and five female rats in each group were evaluated at 3, 6, and 12 months for plasma emodin concentrations; these rats were necropsied at 12 months.

Survival, Body Weights, and Feed Consumption

Survival of exposed males and females was similar to that of the controls. The mean body weights of rats in the 2,500 ppm groups were less than those of the controls beginning at week 2 of the study. Feed consumption by exposed groups was similar to that by the controls throughout the study.

Pathology Findings

Three Zymbal's gland carcinomas were observed in female rats exposed to 2,500 ppm. This incidence exceeded the range observed for current historical controls and was considered an equivocal finding.

At the 6- and 12-month interim evaluations and at 2 years, emodin-related increases in the incidences of renal tubule hyaline droplets occurred in all exposed groups. The incidences of renal tubule pigmentation were significantly increased in all exposed groups of males at 2 years.

There were negative trends in the incidences of mononuclear cell leukemia in male and female rats, and the incidences in the 2,500 ppm groups were significantly decreased. In females exposed to 2,500 ppm, the incidence was below the historical control range; the incidence in males exposed to 2,500 ppm was at the lower end of the historical control range.

2-YEAR STUDY IN MICE

Groups of 60 male mice were fed diets containing 0, 160, 312, or 625 ppm emodin (equivalent to average daily doses of approximately 15, 35, or 70 mg/kg) for 105 weeks. Groups of 60 female mice were fed diets containing 0, 312, 625, or 1,250 ppm emodin (equivalent to average daily doses of approximately 30, 60, or 120 mg/kg) for 105 weeks. Ten male and ten female mice from each group were necropsied at 12 months.

Survival, Body Weights, and Feed Consumption

Survival and mean body weights of exposed males and females were similar to those of the controls. No differences in feed consumption were noted between exposed and control groups.

Pathology Findings

Low incidences of renal tubule adenoma and carcinoma occurred in exposed male mice; these incidences included one carcinoma each in the 312 and 625 ppm groups. Renal tubule neoplasms are rare in male mice, and their presence in these groups suggested a possible association with emodin exposure.

At the 12-month interim evaluation, the severity of nephropathy was slightly increased in males exposed to 625 ppm. Also at 12 months, the severity of nephropathy increased from minimal in the lower exposure groups to mild in females exposed to 1,250 ppm; the incidence in this group was significantly increased compared to the control group. At 2 years, the severities of nephropathy were slightly increased in males exposed to 625 ppm and females exposed to 1,250 ppm. The incidences of nephropathy were significantly increased in all exposed groups of females. At the 12-month interim evaluation, the incidences of renal tubule pigmentation were significantly increased in all exposed groups of males and in females exposed to 625 or 1,250 ppm. The severities increased with increasing exposure concentration. At 2 years, the incidences of renal tubule pigmentation were significantly increased in all exposed groups; severities increased with increasing exposure concentration.

GENETIC TOXICOLOGY

Emodin was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of S9 activation; no mutagenicity was detected in strain TA98, with or without S9. Chromosomal aberrations were induced in cultured Chinese hamster ovary cells treated with emodin, with and without S9. Three separate *in vivo* micronucleus tests were performed with emodin. A male rat bone marrow micronucleus test, with emodin administered by three intraperitoneal injections, gave negative results. Results of acute-exposure (intraperitoneal injection) micronucleus tests in bone marrow and peripheral blood erythrocytes of male and female mice were negative. In a peripheral blood micronucleus test on mice from the 14-week study, negative results were seen in male mice, but a weakly positive response was observed in similarly exposed females.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of emodin in male F344/N rats exposed to 280, 830, or 2,500 ppm. There was *equivocal evidence of carcinogenic activity* of emodin in female F344/N rats based on a marginal increase in the incidence of Zymbal's gland carcinoma. There was *equivocal evidence of carcinogenic activity* of emodin in male B6C3F_1 mice based on a low incidence of uncommon renal tubule neoplasms. There was *no evidence of carcinogenic activity* of emodin in female B6C3F_1 mice exposed to 312, 625, or 1,250 ppm.

Exposure of rats to emodin resulted in increased incidences of renal tubule hyaline droplets and pigmentation in males, increased incidences of renal tubule hyaline droplets in females, and increased severities of renal tubule pigmentation in males and females. Emodin exposure resulted in increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice.

Incidences of mononuclear cell leukemia decreased in male and female rats exposed to 2,500 ppm.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Emodin

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 280, 830, or 2,500 ppm	0, 280, 830, or 2,500 ppm	0, 160, 312, or 625 ppm	0, 312, 625, or 1,250 ppm
Body weights	2,500 ppm group less than control group	2,500 ppm group less than control group	Exposed groups similar to control group	Exposed groups similar to control group
Survival rates	30/50, 21/50, 21/50, 30/50	33/50, 39/50, 35/50, 34/50	41/50, 37/50, 40/50, 43/50	37/50, 39/50, 40/50, 36/50
Nonneoplastic effects	Kidney: renal tubule hyaline droplet (3/50, 45/50, 43/50, 43/50); renal tubule pigmentation (35/50, 47/50, 49/50, 50/50); severity of pigmentation (1.3, 1.8, 1.8, 2.1)	Kidney: renal tubule hyaline droplet (22/49, 49/50, 49/49, 50/50); severity of pigmentation (1.2, 1.4, 2.4, 3.0)	Kidney: renal tubule pigmentation (0/49, 46/50, 50/50, 50/50)	Kidney: renal tubule pigmentation (0/49, 37/50, 48/50, 49/49); nephropathy (22/49, 46/50, 41/50, 48/49)
Neoplastic effects	None	None	None	None
Uncertain findings	None	Zymbal's gland: carcinoma (0/50, 0/50, 0/50, 3/50)	Kidney: renal tubule adenoma (standard evaluation - 0/49, 1/50, 1/50, 0/50; standard and extended evaluations combined - 0/49, 1/50, 1/50, 1/50); renal tubule carcinoma (standard evaluation - 0/49, 0/50, 1/50, 1/50; renal tubule adenoma or carcinoma (standard evaluation - 0/49, 1/50, 2/50, 1/50; standard and extended evaluations combined - 0/49, 1/50, 2/50, 2/50)	None
Decreased incidences	Mononuclear cell leukemia: 28/50, 31/50, 29/50, 18/50	Mononuclear cell leukemia: 14/50, 17/50, 16/50, 3/50	None	None
Level of evidence of carcinogenic activity	No evidence	Equivocal	Equivocal	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive in strain TA100 with S9; negative in strain TA100 without S9; negative with and without S9 in strain TA98			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9			
Micronucleated erythrocytes				
Rat bone marrow *in vivo*:	Negative when administered as intraperitoneal injections			
Mouse bone marrow *in vivo*:	Negative when administered as intraperitoneal injections			
Mouse peripheral blood *in vivo*:	Negative when administered as intraperitoneal injections; negative in males and weakly positive in females when administered in feed for 14 weeks			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on emodin on 21 May 1999 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 21 May 1999, the draft Technical Report on the toxicology and carcinogenesis studies of emodin received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of emodin by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year feed studies were *no evidence of carcinogenic activity* in male F344/N rats and female B6C3F$_1$ mice and *equivocal evidence of carcinogenic activity* in female F344/N rats and male B6C3F$_1$ mice.

Dr. Hecht, a principal reviewer, agreed with the proposed conclusions, but he thought that squamous cell carcinomas of the nose in rats should be considered related to emodin exposure. Because the rat nose, known to be a rich source of cytochrome P450 enzymes, could metabolically activate emodin, he said the possibility of a relationship between the neoplasms to emodin exposure could not be categorically ruled out. Dr. Irwin agreed that the potential for metabolic activation existed, but there were just two animals with nasal neoplasms and there were no other indications of preneoplastic activity such as squamous metaplasia.

Dr. Chatman, the second principal reviewer, agreed with the proposed conclusions. She asked why emodin, a cathartic, did not show laxative effects in the 2-year studies and whether water consumption was monitored. Dr. Irwin said the lack of cathartic effects was a surprise and that water consumption was not specifically monitored. In view of conflicting results on genotoxicity, Dr. Chatman asked if there were any additional studies planned. She noted the first pass effect and need for metabolic activation suggesting a metabolite as the genotoxic form. Dr. Irwin responded that further studies were not planned and that 2-hydroxyemodin, a metabolite, acts as the genotoxin. Dr. Chatman noted increased estrogenic activity of emodin reported in an early study and asked if there was a potential for reproductive problems in women who abuse laxatives. Dr. Irwin said endocrine disruptor screening of emodin would be worthwhile.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions and asked about estrogenic effects of emodin. Dr. Irwin reported a lengthening of the estrous cycle in exposed rats but no changes in morphology in the uterus.

There was considerable discussion as to whether increased incidences of bone marrow hyperplasia and hematopoietic cell proliferation in rats were exposure-related effects or secondary to decreased incidences of mononuclear cell leukemia in male and female rats. Drs. J.R. Hailey and A. Nyska, NIEHS, concluded that these increases were secondary to decreased mononuclear cell leukemia and agreed to add the interpretation in the report. Dr. Medinsky suggested more discussion on the toxicokinetics, noting that low concentrations of emodin in the blood could be due to poor gastrointestinal absorption and/or an extensive first pass effect by the liver in which a majority of the parent compound is metabolized. Dr. Irwin agreed and noted that extensive studies were not done due to limited amounts of the test material and because there was considerable information on metabolism and disposition in the literature. Dr. Cullen observed that emodin may not be a cathartic in rodents due to a different gut structure from humans and certainly different reabsorption capability in colonic function. Dr. Bailer questioned the lack of attention to several small, apparently exposure concentration related increases in neoplasms, such as Harderian gland carcinomas. Dr. Hailey explained that NTP considers analysis of benign and malignant neoplasms combined to be the most relevant when looking at tumorigenic effects, and in doing this with the Harderian gland, there was neither a positive trend nor pairwise differences. Dr. Chatman asked if possible antileukemic effects of emodin were being evaluated. Dr. Irwin replied that emodin, as well as some derivatives, was being evaluated for human use as an anticancer agent.

Dr. Hecht moved that the Technical Report on emodin be accepted with revisions discussed and with the conclusions as written for male rats and female *mice, no evidence of carcinogenic activity*, and for female rats and male mice, *equivocal evidence of carcinogenic activity*. Dr. Chatman seconded the motion, which was accepted unanimously with nine votes.